



# **Ozařování trombocytových přípravků, pro a proti**

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# Conflict of interest

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## Cíl přednášky

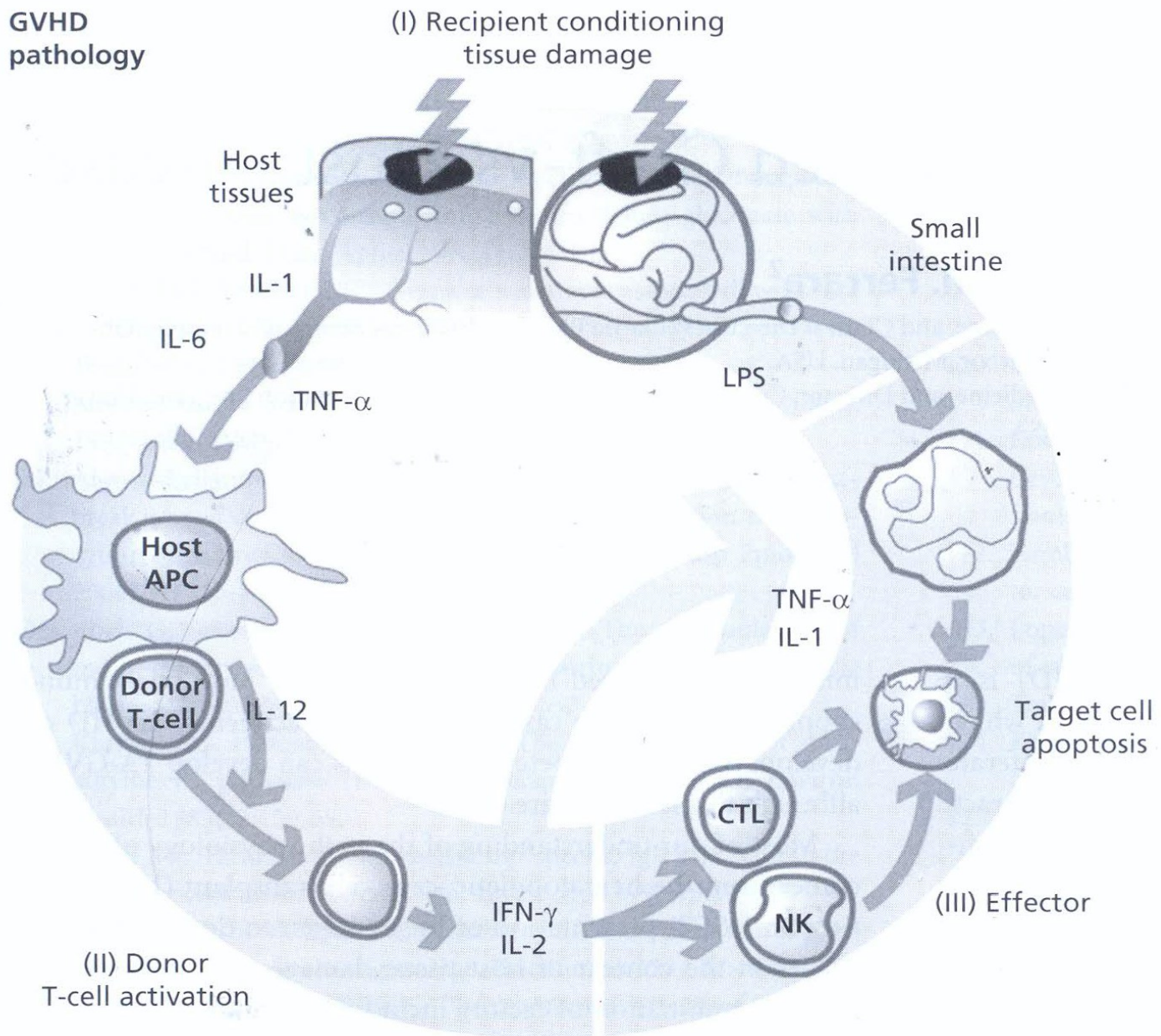
- **TA-GvHD**
- **Ozařování - princip**
- **Metody, intervaly ozařování TP**
- **Ozařování trombocytových přípravků**
- **Indikace ozařování**



## **TA-GvHD**

- **Vzácná komplikace, závažné postižení pac.**
- **Smrtnost 80 – 90 %**
- **Proliferace dárcovských T lymfocytů v imunokompromitovaném organismu příjemce**
- **Efekt dávky**

# GVHD pathology





## **Příznaky**

- **Za 2 – 30 dnů po transfuzi**
- **Horečka**
- **Kůže - difuzní makulopapulózní vyrážka (rash),  
progrese až erythroderma nebo deskvamace**
- **Játra – hepatomegalie, zánět**
- **GIT - nauzea, zvracení, průjem**
- **Kostní dřeň - cytopenie**



# **Diagnóza TA-GvHD**

- **Klinická**
- **Biopsie kůže**
- **Průkaz chimerismu**
  
- **Cave!**
- **Slabé formy**
- **Výskyt u nekompromitovaných pacientů (HLA haplotyp)**



# Prevence

- **Ozařování TP, P-I**
- **Princip funkce**
- **Ozařovače cesiové ( $\gamma$ ), rentgenové (x)**



## Ozařování TP – náhradní řešení

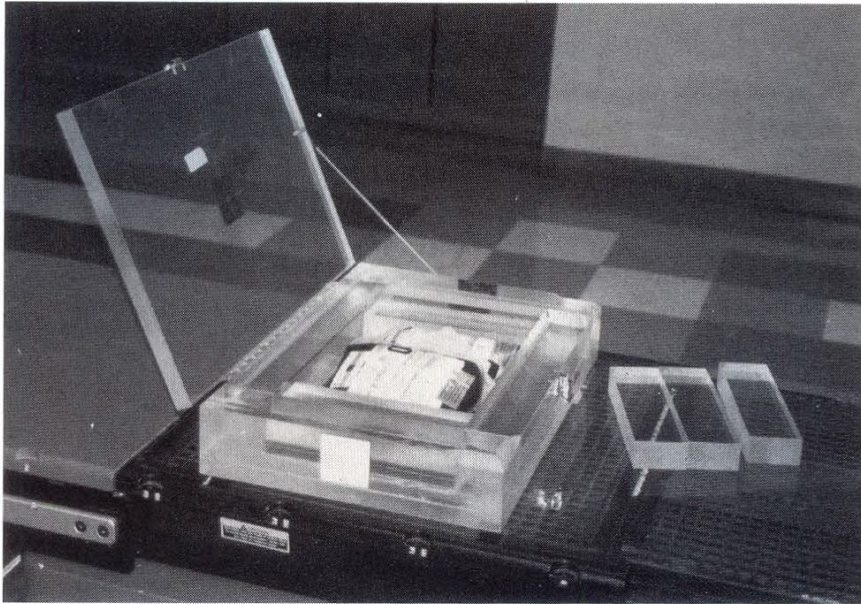


Fig. 2. Blood irradiation box in use with blood components in well. The acrylic spacers adjacent to the box are used to eliminate any voids in the box after the blood components are inserted.

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A Patton, Transfusion, 2001

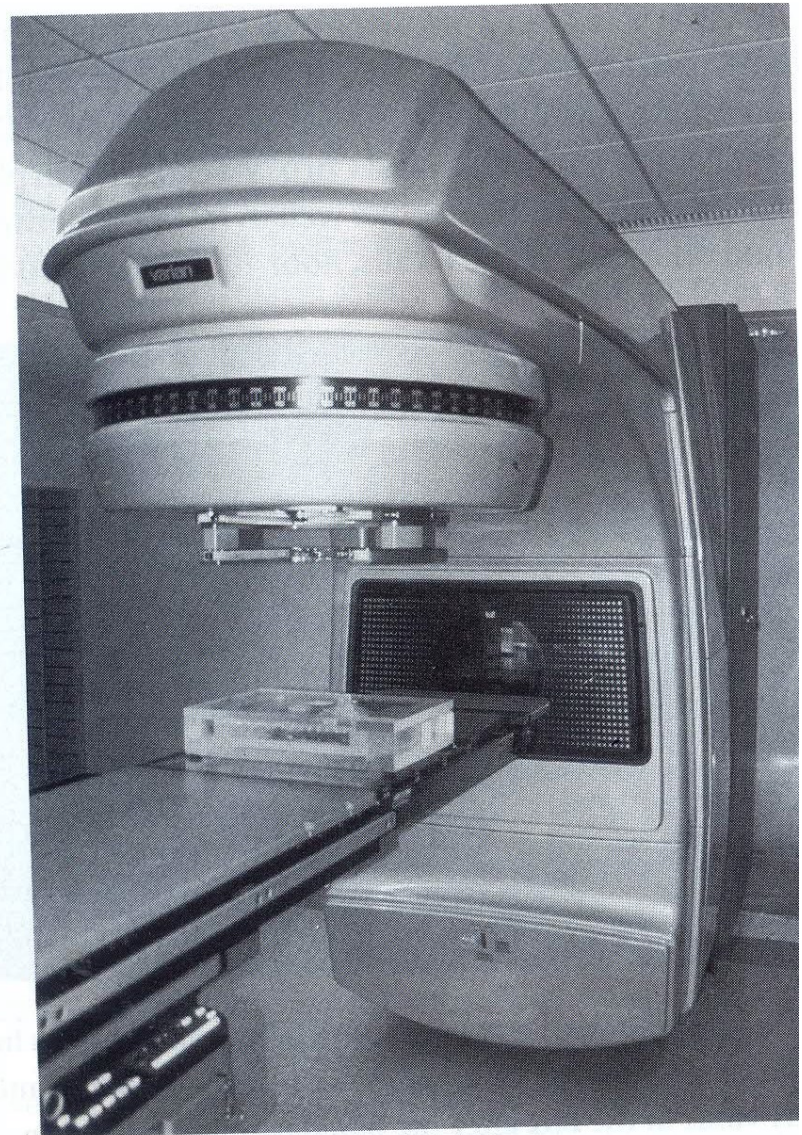


Fig. 3. Blood irradiation box in use.



# **Pravidla pro ozařování TP**

- **Erytrocyty:**
  - **Min. dávka 25 Gy v každém místě ozařovacího pole (max. 50 Gy)**
  - **Podat co nejdříve po ozáření, nejdéle do 14 dnů**
  - **Do 28. dne od odběru (v USA odlišně)**
  - **IUT (povinně, <24 hod.), výměnná t. (ano, pokud není nebezpečí z prodlení), novorozenci (indik.)**
- **Granulocyty: vždy ozářit**
- **Trombocyty: ?**





# Ozařování trombocytů

- **Dávka** (N Luban, Transfusion, 2000)
  - 5 Gy: 2,09 log, 15 Gy: 3,96 log - detekovány živé Ly
  - 25 Gy: >4,86 log, žádné viabilní Ly
- **Srovnání ozářených a neozářených TP trombocytů**
- **Srovnání ozářených TP trombocytů ( $\gamma$  vs. rtg)**

# Ozářené vs neozářené TP trombocytů z B-C

**Table 1** *In vitro* parameters of leukoreduced platelet concentrates in plasma, irradiated with 25 Gy on day 1

|                               | Reference   | Irradiated  | P-value |
|-------------------------------|-------------|-------------|---------|
| Day 1                         |             |             |         |
| Volume, ml                    | 362 ± 13    | 363 ± 15    | 0.92    |
| Platelets, × 10 <sup>9</sup>  | 411 ± 35    | 394 ± 40    | 0.15    |
| Leucocytes, × 10 <sup>6</sup> | 0.04 ± 0.04 | 0.05 ± 0.05 | 0.52    |
| Red cells, × 10 <sup>9</sup>  | 0.13 ± 0.08 | 0.14 ± 0.11 | 0.45    |
| Day 8                         |             |             |         |
| Swirl                         | 2.7 ± 0.6   | 2.6 ± 0.8   | 0.34    |
| pH                            | 6.85 ± 0.19 | 6.73 ± 0.34 | 0.07    |
| pO <sub>2</sub> , mmHg        | 52 ± 13     | 47 ± 16     | 0.48    |
| pCO <sub>2</sub> , mmHg       | 45 ± 3      | 51 ± 22     | 0.40    |
| Bicarbonate, mmol/l           | 7.6 ± 2.3   | 8.0 ± 2.1   | 0.32    |
| Glucose, mmol/l               | 11.6 ± 2.2  | 10.2 ± 3.6  | 0.09    |
| Lactate, mmol/l               | 15.6 ± 3.5  | 17.5 ± 5.7  | 0.12    |
| CD62 expression, %            | 20 ± 7      | 23 ± 12     | 0.33    |
| Morphology                    | 223 ± 26    | 194 ± 56    | 0.06    |

Results are expressed as mean ± standard deviation (n = 12).

**Table 2** *In vitro* parameters of leucoreduced platelet concentrates in plasma, irradiated with 25 Gy on day 5

|                               | Reference   | Irradiated  | P-value |
|-------------------------------|-------------|-------------|---------|
| Day 1                         |             |             |         |
| Volume, ml                    | 373 ± 16    | 373 ± 12    | 0.82    |
| Platelets, × 10 <sup>9</sup>  | 370 ± 28    | 372 ± 33    | 0.76    |
| Leucocytes, × 10 <sup>6</sup> | 0.03 ± 0.02 | 0.02 ± 0.02 | 0.03    |
| Red cells, × 10 <sup>9</sup>  | 0.14 ± 0.08 | 0.13 ± 0.08 | 0.51    |
| Day 8                         |             |             |         |
| Swirl                         | 2.4 ± 0.8   | 2.4 ± 0.7   | 0.87    |
| pH                            | 6.99 ± 0.10 | 6.92 ± 0.23 | 0.22    |
| pO <sub>2</sub> , mmHg        | 49.3 ± 8.3  | 47.9 ± 8.7  | 0.72    |
| pCO <sub>2</sub> , mmHg       | 41.4 ± 2.6  | 44.1 ± 4.5  | 0.10    |
| Bicarbonate, mmol/l           | 13.5 ± 1.4  | 13.0 ± 2.7  | 0.28    |
| Glucose, mmol/l               | 14.0 ± 2.2  | 14.9 ± 3.8  | 0.21    |
| Lactate, mmol/l               | 9.5 ± 1.7   | 9.7 ± 1.2   | 0.10    |
| CD62 expression, %            | 19 ± 4      | 22 ± 11     | 0.23    |
| Morphology                    | 212 ± 27    | 195 ± 59    | 0.15    |

Results are expressed as mean ± standard deviation (n = 12).

PF van der Meer, Vox Sang 2005 (srovnatelný úbytek glukózy 0,09 μmol/den/10<sup>11</sup> trombo)

# Ozářené vs neozářené TP trombocytů (afer.)

**TABLE 1. Changes in blood gases and metabolic variables during storage of irradiated and nonirradiated (control) PCs\***

| Variable               | PC type    | Day 0        | Day 1         | Day 5         | Day 7        |
|------------------------|------------|--------------|---------------|---------------|--------------|
| pH                     | Irradiated | 7.07 ± 0.06‡ | 7.33 ± 0.06†‡ | 7.28 ± 0.08†‡ | 7.17 ± 0.11† |
|                        | Control    | 7.09 ± 0.10‡ | 7.33 ± 0.05†‡ | 7.28 ± 0.06†‡ | 7.17 ± 0.10† |
| pO <sub>2</sub> (kPa)  | Irradiated | 16.1 ± 2.1   | 16.0 ± 2.4‡   | 16.7 ± 1.8    | 17.2 ± 1.7   |
|                        | Control    | 16.4 ± 2.2‡  | 16.1 ± 1.7‡   | 16.8 ± 1.8    | 17.4 ± 1.3†  |
| pCO <sub>2</sub> (kPa) | Irradiated | 8.2 ± 1.2‡   | 4.3 ± 0.5†‡   | 3.1 ± 0.4†‡   | 2.6 ± 0.4†§  |
|                        | Control    | 8.1 ± 1.6‡   | 4.3 ± 0.3†‡   | 3.1 ± 0.4†‡   | 2.8 ± 0.3†§  |
| Glucose (mmol/L)       | Irradiated | 17.7 ± 2.2‡  | 16.8 ± 2.4†‡  | 12.9 ± 2.7†‡  | 10.5 ± 2.8†  |
|                        | Control    | 17.7 ± 2.2‡  | 17.0 ± 2.1†‡  | 12.9 ± 2.6†‡  | 10.5 ± 2.8†  |
| Lactate (mmol/L)       | Irradiated | 2.8 ± 0.8‡   | 4.0 ± 1.0†‡   | 11.3 ± 2.2†‡  | 16.3 ± 3.0†§ |
|                        | Control    | 2.8 ± 0.9‡   | 3.9 ± 0.9†‡   | 11.0 ± 2.2†‡  | 15.8 ± 3.0†§ |
| LDH (μkat/L)           | Irradiated | 2.4 ± 0.4‡   | 2.5 ± 0.5‡    | 3.0 ± 1.1†    | 3.2 ± 1.4†   |
|                        | Control    | 2.5 ± 0.4‡   | 2.5 ± 0.4‡    | 3.4 ± 2.3     | 3.3 ± 1.5†   |

\* Data are given as mean ± SD for n = 20 except for pO<sub>2</sub> and pCO<sub>2</sub> for both irradiated and nonirradiated PCs on Day 0 where n = 19 due to technical difficulties. Statistical differences between storage days are indicated separately for irradiated and nonirradiated PCs.

† p < 0.05 vs. Day 0.

‡ p < 0.05 vs. Day 7.

§ p < 0.05 for irradiated vs. non-irradiated PCs.



## Ozářené vs neozářené TP trombocytů

**TABLE 2. Flow cytometry analyses of irradiated and nonirradiated (control) PCs during storage\***

| Variable                      | PC type    | Day 1      | Day 5       | Day 7      |
|-------------------------------|------------|------------|-------------|------------|
| <b>GPIb</b>                   |            |            |             |            |
| Percent                       | Irradiated | 87 ± 5     | 88 ± 5      | 87 ± 4     |
|                               | Control    | 86 ± 6     | 88 ± 5      | 87 ± 5     |
| MFI (AU)                      | Irradiated | 32.5 ± 6.3 | 34.5 ± 7.0‡ | 31.3 ± 6.2 |
|                               | Control    | 33.2 ± 7.0 | 34.7 ± 8.3‡ | 32.0 ± 8.4 |
| <b>Spontaneous P-selectin</b> |            |            |             |            |
| Percent                       | Irradiated | 15 ± 8‡§   | 25 ± 9†‡    | 32 ± 10†   |
|                               | Control    | 13 ± 7‡§   | 24 ± 9†‡    | 32 ± 10†   |
| MFI (AU)                      | Irradiated | 1.2 ± 0.6‡ | 1.6 ± 0.5†‡ | 2.1 ± 0.5† |
|                               | Control    | 1.1 ± 0.6‡ | 1.7 ± 0.6†‡ | 2.0 ± 0.5† |
| <b>TRAP-6 P-selectin</b>      |            |            |             |            |
| Percent                       | Irradiated | 85 ± 5     | 85 ± 5      | 83 ± 6     |
|                               | Control    | 84 ± 6     | 85 ± 6      | 83 ± 5     |
| MFI (AU)                      | Irradiated | 9.3 ± 1.9‡ | 7.8 ± 1.8†‡ | 7.0 ± 1.5† |
|                               | Control    | 9.4 ± 2.3‡ | 8.0 ± 1.7†‡ | 6.9 ± 1.5† |

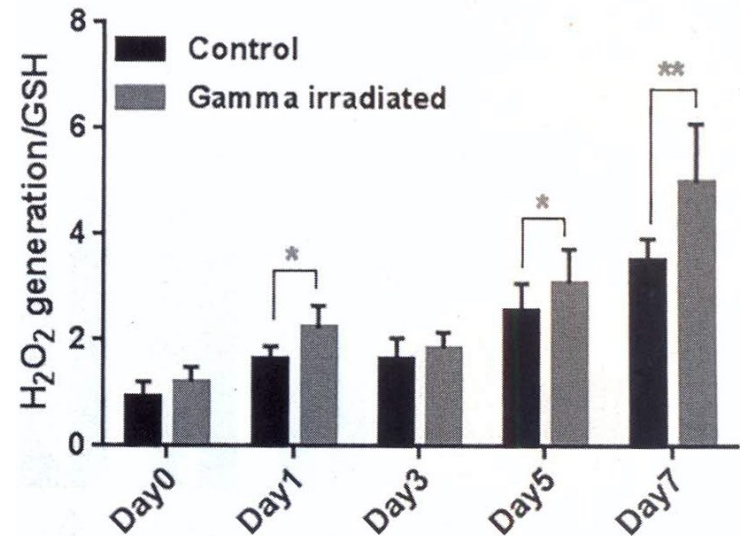
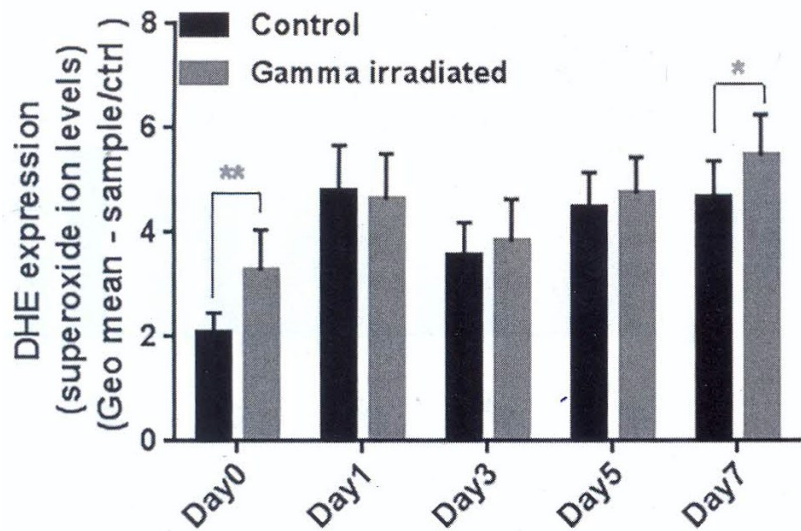
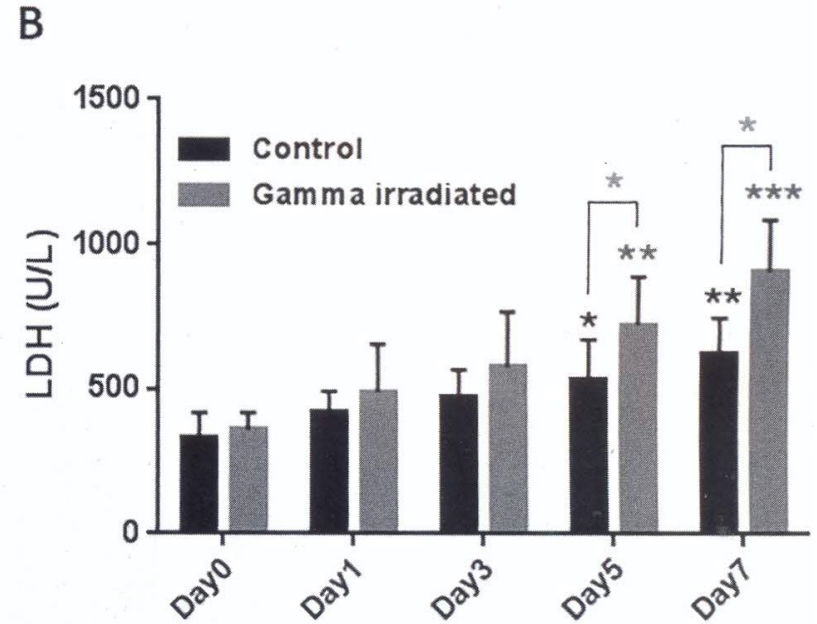
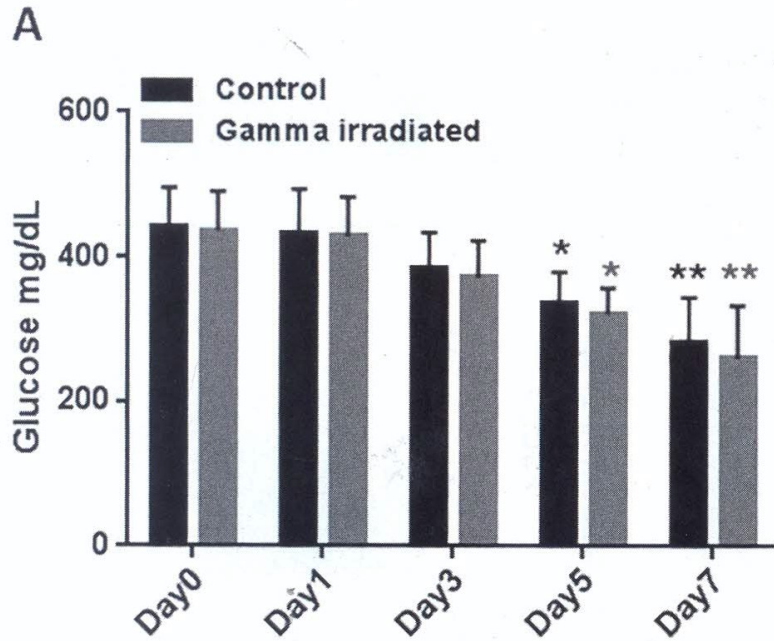
\* Data are given as mean ± SD for n = 20. Spontaneous and TRAP-6-induced P-selectin expression as well as GPIb expression are presented as percentage of expressing cells and MFI. Statistical differences between storage days are indicated separately for irradiated and nonirradiated PCs.

† p < 0.05 vs. Day 1.

‡ p < 0.05 vs. Day 7.

§ p < 0.05 for irradiated vs. nonirradiated PCs.

# Ozářené vs neozářené TP trombocytů (PRP Plt, LD?)





## Ozařování trombocytů – srovnání y a rtg (afer.)

TABLE 1 Characterization of metabolic parameters in platelet components over storage<sup>a</sup>

| Pooled platelets    |             |              |             |             |                  |             |             |         |                             |
|---------------------|-------------|--------------|-------------|-------------|------------------|-------------|-------------|---------|-----------------------------|
| Group<br>Parameter  | Day 1       | X-irradiated |             |             | Gamma-irradiated |             |             | p-value | Non-<br>irradiated<br>Day 5 |
|                     |             | Day 2        | Day 5       | Day 7       | Day 2            | Day 5       | Day 7       |         |                             |
| MPV (fL)            | 4.7 ± 0.2   | 4.7 ± 0.2    | 4.5 ± 0.2   | 4.3 ± 0.2   | 4.7 ± 0.2        | 4.5 ± 0.2   | 4.4 ± 0.2   | .660    | 4.4 ± 0.3                   |
| Glucose (mmol/L)    | 5.5 ± 0.4   | 5.1 ± 0.4    | 3.9 ± 0.5   | 3.1 ± 0.6   | 5.1 ± 0.5        | 4.0 ± 0.5   | 3.1 ± 0.6   | .931    | 4.1 ± 0.9                   |
| Lactate (mmol/L)    | 5.7 ± 0.7   | 6.0 ± 0.9    | 7.9 ± 1.0   | 9.7 ± 1.1   | 6.1 ± 1.0        | 7.9 ± 1.2   | 9.2 ± 1.1   | .328    | 8.0 ± 1.6                   |
| pH <sub>22°C</sub>  | 7.11 ± 0.05 | 7.21 ± 0.08  | 7.28 ± 0.08 | 7.27 ± 0.07 | 7.19 ± 0.07      | 7.27 ± 0.06 | 7.26 ± 0.07 | .669    | 7.26 ± 0.09                 |
| Apheresis platelets |             |              |             |             |                  |             |             |         |                             |
| Group<br>Parameter  | Day 1       | X-irradiated |             |             | Gamma-irradiated |             |             | p-value | Non-<br>irradiated<br>Day 5 |
|                     |             | Day 2        | Day 5       | Day 7       | Day 2            | Day 5       | Day 7       |         |                             |
| MPV (fL)            | 4.1 ± 0.4   | 4.0 ± 0.4    | 3.9 ± 0.3   | 3.9 ± 0.3   | 4.0 ± 0.4        | 3.9 ± 0.3   | 3.9 ± 0.3   | .652    | 4.0 ± 0.4                   |
| Glucose (mmol/L)    | 6.4 ± 0.7   | 5.9 ± 0.8    | 4.4 ± 0.8   | 3.3 ± 0.9   | 5.9 ± 0.8        | 4.4 ± 0.9   | 3.3 ± 0.9   | .979    | 4.4 ± 1.0                   |
| Lactate (mmol/L)    | 2.8 ± 1.1   | 3.6 ± 1.2    | 6.5 ± 1.5   | 8.5 ± 1.9   | 3.6 ± 1.1        | 6.5 ± 1.5   | 8.7 ± 1.7   | .854    | 6.8 ± 1.6                   |
| pH <sub>22°C</sub>  | 7.28 ± 0.09 | 7.37 ± 0.07  | 7.44 ± 0.09 | 7.42 ± 0.08 | 7.37 ± 0.09      | 7.42 ± 0.10 | 7.41 ± 0.09 | .657    | 7.40 ± 0.11                 |

<sup>a</sup>Values shown are mean ± SD; n = 20 for X- and gamma-irradiated; n = 10 for non-irradiated reference units.

<sup>a</sup>p-value obtained using two-way repeated measures ANOVA.





# Indikace ozařování TP

## *Irradiation Policy at RCH (Roayal Childrens H. Melbourne)*

At RCH a universal blood irradiation policy applies for patients in the following units: PICU, NICU and Children's Cancer Centre. Although not all patients in these units are at risk of TA-GVHD, this policy ensures patients who require irradiated products are not missed.

(Note - haematopoietic stem cells and donor lymphocytes **must not** be irradiated).

In addition blood is irradiated at RCH in the following circumstances:

1. Infants at R.C.H. under 4 months of age
2. Cardiac patients with potential T-cell immunodeficiency
3. Immuno-compromised patients i.e.
  - 3.1. Severe Combined Immune Deficiency (SCID), Common Variable Immune Deficiency (CVID)
  - 3.2. Malignancy
  - 3.3. Transplant recipients (bone marrow)
  - 3.4. Haematology/Oncology patients
4. Neonatal exchange transfusions
5. Directed donations



# Indikace ozařování TP

Prevention of Transfusion Associated Graft Versus Host Disease (TA-GVHD) in cellular blood products given to

- Patients receiving products from first-degree relatives\* or HLA-matched donors regardless of patient's immune status
- *Immunocompromised patients such as*
  - Infants (particularly premature) up to 4, 6, or 12 months depending on institutional policy
  - Intrauterine transfusion\* and/or neonatal exchange transfusion recipients
  - Congenital immunodeficiency disorders of cellular immunity (i.e., SCID, DiGeorge)\*
  - Hematopoietic progenitor cell transplant recipients\*
  - Hodgkin's disease\*, Leukemia, or Lymphoma patients
  - Patients treated with nucleoside analogs (i.e., fludarabine)
  - Patients requiring granulocyte transfusions\*
  - Solid Organ Tumor patients undergoing intense chemotherapy (controversial and not universal)
  - Solid organ transplant recipient (controversial and not universal)
  - Aplastic anemia with severe lymphocytopenia (controversial and not universal)
- Irradiation of thawed plasma and cryoprecipitate are not necessary as they have never been associated with TA-GVHD. Fresh liquid plasma (never frozen) may have a small amount of viable lymphocytes and should be irradiated if the patient has indications for irradiated cellular blood products.



# Indikace ozařování TP

**Indikace ozáření erytrocytových a trombocytových přípravků z důvodu snížení rizika potransfuzní reakce štěpu proti hostiteli (TA-GVHD)**

- Pacienti s primárním imunodeficitem,
- Pacienti se získaným imunodeficitem, např.
  - Pacienti léčení imunosupresivy nebo cytostatiky,
  - Pacienti před, v průběhu a po alogenní transplantaci krvetvorných buněk a solidních orgánů, tj. ještě po dobu profylaxe event. léčby GVHD,
  - Pacienti před, v průběhu a ještě 3 měsíce po autologní transplantaci krvetvorných buněk (včetně léčby CAR-T buňkami), tj. po dobu než dojde k rekonstituci imunitních funkcí,
  - Pacienti, kteří podstoupili léčbu purinovými analogy (např. fludarabin, cladribin, bendamustin) nebo alemtuzumabem (celoživotně),
  - Pacienti s Hodgkinovým lymfomem (celoživotně),
  - Pacienti před a dále po dobu 6 měsíců po podání antithymocytárního globulinu.
- Intrauterinní transfuze, transfuze u všech novorozenců,
- Transfuze pediatrickým pacientům do 6 let věku (nad 6 let dle indikace lékaře),
- Transfuzní přípravky získané od geneticky příbuzných dárců pacienta,
- Transfuze HLA kompatibilních transfuzních přípravků.



# **Indikace ozařování TP**

- **Nejednotné**
- **Japonsko povinné ozařování TP od 2001**
  
- **Ideální standard**
  - **Zahrnuje absolutní indikace snižující riziko TA-GvHD**
  - **Lze přidat další možné indikace dle rozhodnutí instituce**
  - **Doporučení konzultovat lékaře krevní banky**





## **Závěr**

- **Ozařovat TP trombocytů indikovaným pacientům**
- **TP trombocytů lze ozařovat v den výroby**
- **Doporučení STL pro ozařování TP v přípravě**